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Research Article

Invasive mosquitofish impact a threatened toothcarp through water quality impairment and resource competition

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Abstract

Invasive non-native species are an important cause of biodiversity loss, particularly in fresh waters. The mosquitofish *Gambusia holbrooki* are among the world's worst invasive species: they have caused extirpations of native species and are known to sometimes cause trophic cascades and ecosystem effects. This invasive species is also known to impact threatened fishes such as the Spanish toothcarp (*Aphanius iberus*), which is endemic to Mediterranean Spain. However, it is unclear if the impact of mosquitofish on many fishes is more through resource competition, agonistic interactions or predation, and how often mosquitofish cause trophic cascades. To clarify these questions, we performed a 48-day mesocosm experiment in eutrophic conditions to test for interspecific effects and clarify the impact mechanism using six treatments: the two fish species alone each at two densities, and the two fish species mixed or separated with a net that prevented direct interactions among them. We observed clear fish treatment effects on several variables. At low initial fish densities, the population growth rate of mosquitofish was orders of magnitude greater than that of the Spanish toothcarp, likely contributing to its invasive success and ecological impact. At high fish densities, turbidity, chlorophyll *a* concentration and daytime dissolved oxygen percentage increased, whereas total phosphorus decreased; crucially, the trophic cascade caused by mosquitofish was stronger than that by toothcarp. The experiment also demonstrated that the interspecific effects of mosquitofish on toothcarp were more important than those of intraspecific competition. The invasive species produced effects on population growth rate, size structure, and fish condition (mass-length relationship) of toothcarp. Effects on population growth rate of toothcarp seem more caused by resource competition, whereas impacts on size structure and condition seem also caused by more direct interactions. The diversity of effects of mosquitofish underscores the difficulty of predicting the impact of invasive species. Our study further provides an approach to differentiate the effects of resource competition from other more direct ecological interactions and so to clarify the impact mechanism of aquatic invasive species.

Key words: Interference competition, introduced non-native species, mesocosm experiment, trophic cascade, turbidity

Introduction

Freshwater ecosystems are fundamental for humankind and rich in biodiversity, but also highly impacted by human-mediated alterations, including damming, water abstraction and pollution, and invasive non-native species (e.g. Dudgeon et al. 2006; Albert et al. 2021). Invasive species are one of the main drivers of biodiversity loss in fresh waters (Clavero and García-Berthou 2005; Vitule et al. 2009), but sometimes their effects are difficult to isolate from other anthropogenic impacts that are the direct cause of native decline (Sayer et al. 2025). Invasive species can directly impact native biodiversity through diverse mechanisms, including predation, competition, or whole ecosystem modification, among others (Bohn and Amundsen 2001; Alcaraz et al. 2008; Cucherousset and Olden 2011; Catford et al. 2022).

Two closely related, often confounded mosquitofishes (*Gambusia affinis* and *Gambusia holbrooki*) have been introduced worldwide, allegedly for controlling mosquitoes as vectors of malaria and other diseases (García-Berthou et al. 2005; Azevedo-Santos et al. 2017), and are considered among the worst invaders (Lowe et al. 2000), often causing declines and extirpations of small fish and amphibians (Alcaraz et al. 2008; Henkanaththegedara and Stockwell 2011). These North American mosquitofishes are well known to cause trophic cascades by depleting zooplankton and increasing phytoplankton and thus turbidity (Hurlbert et al. 1972; Hurlbert and Mulla 1981; Rettig and Smith 2021). They impact native fishes through resource competition (i.e. depletion of food resources), predation and interference competition (e.g. agonistic behaviour) (Rogowski and Stockwell 2006; Pyke 2008; Thompson et al. 2012; Tsang and Dudgeon 2021), but the importance of the different mechanisms is unclear. For instance, in some cases the main mechanism seems to be predation on eggs and young-of-the-year fish (Meffe et al. 1983; Meffe 1985), although this is often not detected in gut contents (Alcaraz et al. 2008; Carmona-Catot et al. 2013). Resource and interference competition is also known to depend on salinity and temperature in the eastern mosquitofish (Meffe 1985; Alcaraz et al. 2008; Carmona-Catot et al. 2013), which is the main species introduced in Europe, northern Africa, Australia and part of Asia (Pyke 2008).

Cyprinodontiform fishes such as the Old World toothcarps (Aphaniidae) are often threatened and affected by invasive *G. holbrooki*. A number of experiments have shown impacts of the mosquitofish on the Spanish toothcarp, *Aphanius iberus* (= *Apricaphanius iberus*), which is considered endangered and protected by Spanish law (Oliva-Paterna et al. 2006). Some of these investigations did not observe agonistic interactions but suggested that the main mechanism was resource competition (Caiola and de Sostoa 2005); many others observed both resource and interference competition (Carmona-Catot et al. 2013; Magellan and García-Berthou 2015, 2016), whereas only Rincón et al. (2002) also observed predation on young-of-the-year toothcarp. Moreover, while many experiments have tested the ecosystem effects of *G. affinis*, fewer have considered *G. holbrooki*. In the latter case, the small number of studies relating to Mediterranean systems found depletion of zooplankton but no (Angeler et al. 2002; Cardona 2006) or weak (Romo and Villena 2005) cascading effects on phytoplankton and nutrients, despite this fish species having very high nutrient excretion rates (Barton et al. 2023).

The endemic toothcarp is limited to a few populations in Mediterranean coastal lagoons (Gonzalez et al. 2018; Nester et al. 2025), which are particularly vulnerable to eutrophication caused by agricultural practices (Badosa et al. 2007; Ejarque et al.

2016). As it is unclear if indirect ecosystem effects mediate the interactions between it and *G. holbrooki*, we conducted a mesocosm experiment to: i) test the effects of invasive mosquitofish on toothcarp and water quality under eutrophic conditions; and ii) disentangle the impact mechanism of mosquitofish on toothcarp. For the first objective we tested the effects of mosquitofish on population growth rate, size structure, and fish condition (mass-length relationship) of toothcarp and compared the effects of both species on water quality. For the latter objective, we used different treatments (species alone, mixed or separated with a fine mesh barrier): a treatment with the two species in the same mesocosm separated with a mesh allowed only resource (or exploitation) competition of mosquitofish on toothcarp, whereas when both species where mixed, predation and interference competition (agonistic interactions) by the invasive species was also possible. We hypothesized that the interspecific effects of mosquitofish on toothcarp might be more important than those of intraspecific competition and that multiple impact mechanisms might be present.

Methods

The experiment was carried out for 48 days at an official conservation centre (Centro Acuícola de El Palmar: 39°17'45"N,0°20'00"W). The centre is a public facility in charge of the regional toothcarp conservation program and is located within the protected Albufera of Valencia Natural Park. The centre performs captive breeding and releases in nature of threatened species (including the toothcarp), among other tasks. The Albufera of Valencia is an oligohaline coastal lagoon of 21 km^2 (Molner et al. 2025) with an average depth of 1 m and connected to the sea through three artificial canals built for rice farming (Soria et al. 2000). Intensive rice farming in the Natural Park contributes to the eutrophication of the lagoon, which shows annual averages of around 95–180 $\mu g\ L^{-1}$ for chlorophyll *a* and 340 $\mu g\ L^{-1}$ for total phosphorus (Onandia et al. 2015; Soria 2006; Soria et al. 2021). The hypertrophic conditions of the lagoon result in marked daily dissolved oxygen oscillations with anoxia events occurring during warm, dry periods (Onandia et al. 2015). The average annual water temperature in the lagoon is about 19 °C, whereas the average annual air temperature and rainfall in the area are 18.3 °C and 472 mm, respectively (Soria et al. 2021).

Six months before the experiment, the toothcarp were caught from the Marjal de los Moros marsh population (Valencia) and acclimated in semi-natural ponds protected with nets at the conservation centre. The outdoor ponds were filled with water from the Albufera channels to a depth of 25 cm and stocked with the local macrophyte *Helosciadium* (=*Apium*) *nodiflorum*. One week before the start of the experiment, mosquitofish were captured using dipnets from Albufera channels and acclimated in tanks with constant aeration at the conservation centre. Until the start of the experiment, fish species were fed daily with pellets and natural zooplankton from the Albufera lagoon.

One month before the experiment, the mesocosms were filled with water from the Albufera channels to a depth of 45 cm (volume ~680 L) and sheltered with nets to avoid avian predation. As a refuge for fish, two pot plants of the macrophyte *H. nodiflorum* were provided per mesocosm. The polyester mesocosms (1.4 m long × 1.08 m wide × 0.9 m deep) were allowed to settle for one month to enable natural colonisation by algae and invertebrates. Photographs and a schematic overview of the experiment are given in the Suppl. material 1: figs S1, S2.

Experimental design

On May 31, we started the experiment by measuring total length of fish (to the nearest mm) (Suppl. material 1: fig. S2, tables S1, S2) and randomly allocating the specimens to six treatments: eight toothcarp (hereafter, T_low); eight mosquitofish (M_low); thirty-six toothcarp (T_high); thirty-six mosquitofish (M_high); thirty-six toothcarp and thirty-six mosquitofish (Mixed); and thirty-six toothcarps and thirty-six mosquitofish separated with a net (Net). The two fish densities (low = 5 fish/m^2; high = 32 fish/m^2) were representative of wild populations during spring and summer seasons (e.g. Zulian et al. 1995; Badosa et al. 2007; Alcaraz et al. 2008) and are similar to those employed in previous mesocosms experiments with mosquitofish (Thompson et al. 2012; Goodchild and Stockwell 2016). The Net treatment, with a mesh size of 1.8 × 1.6 mm, allowed the passage of plankton but not of fish through the holes (see also e.g. Mamani et al. 2019); it thus avoided possible predation or aggression of mosquitofish on toothcarp and informs on the role of resource competition alone between the two species. By contrast, the Mixed treatment informs about the overall effects of interspecific predation and resource and interference competition. The toothcarp sex ratio was approximately 1:1, similar to that of wild populations (Vargas and De Sostoa 1996; Oliva-Paterna et al. 2006). The sex ratio of mosquitofish ranged from 1:1 to 2:3, because females were more abundant during the sampling, as it is frequent in wild populations (Vargas and De Sostoa 1996; Fryxell et al. 2015). Treatments had four replicates and were spatially interspersed (Suppl. material 1: fig. S1) to prevent confusion of treatments with spatial gradients (Hurlbert 1984).

During the experiment, we measured the physical and chemical variables (pH, redox potential, dissolved oxygen, conductivity, turbidity, and water temperature) weekly around 10 am-1 pm using a multiparametric probe (HI9829, Hanna Ltd.). Fish were monitored once every other day to check for possible mortality and dead fish were removed but not replaced.

On 13 July, after observing some fish mortality we terminated the experiment following the animal welfare statement approved by the Experimental Ethics Committee of the Autonomous University of Barcelona. We already anticipated high fish mortality rates in the 1+ age class as most individuals of both species do not reach this age and die after reproduction, which occurs from March to September (Fernandez-Delgado et al. 1988; Fernández-Delgado 1989; García-Berthou and Moreno-Amich 1993).

At the end of the experiment, we assessed differences among treatments in the nutrient concentrations of total nitrogen (TN), total phosphorus (TP), ammonium (NH_4^+), nitrite (NO_2^-), total organic carbon (TOC), and planktonic chlorophyll *a*. We collected 0.5 L of water at each mesocosm and stored below 4 °C until nutrients analysis. We filtered between 0.2 and 1 L of water per mesocosm through a Whatman GF/C filter to measure planktonic chlorophyll *a*. The filters were wrapped in aluminium foil and frozen until analysis. At the laboratory, the filters were thawed and 10 ml of 90% acetone was added. The content was homogenized with an ultrasonic bath and then extracted for 16 to 24 hours at 4 °C in the dark. After extraction, the solutions were passed through a glass fibre Whatman GF/C filter. We repeated the extractions up to three times to ensure the complete extraction of chlorophyll *a* from the filters. The absorbance of the extract was read using a 50 µm cuvette in the spectrophotometer at 630, 647, 664, and 750 wavelengths. Phytoplankton biomass was indirectly measured as chlorophyll *a*, following the Jeffrey and Humphrey

(1975) equation for mixed phytoplankton populations. Nutrients were measured by conventional spectrophotometric and chromatographic techniques.

After the water was collected, the tanks were emptied, and the toothcarp and mosquitofish were counted and measured alive for total length (to the nearest millimetre with a millimetre paper) and mass (to the nearest 0.1 mg with a precision balance). The native toothcarp were preserved at the conservation centre to be released to its original population. Mosquitofish were euthanized using an overdose of MS-222 (250 mg L^{-1} of tricaine) following the Spanish legislation for invasive alien species.

Population growth rates were estimated using the equation $\lambda = \left(\frac{N_t}{N_0}\right)^{\frac{1}{t}}$, where λ is the yearly finite rate of increase, N_t is the abundance at the end of the experiment, N_0 the initial abundance, and t the duration of the experiment in years.

Statistical analyses

We first assessed the overall effect of treatments over time on water quality (i.e. turbidity, dissolved oxygen, conductivity, temperature and redox potential) using principal response curve analysis (PRC), which is a special case of redundancy analysis (RDA). PRC is a multivariate technique used to analyse differences between treatments that are measured repeatedly over time (Van den Brink and Ter Braak 1999; Van den Brink et al. 2003). PRC generates a graphical display with time as a horizontal line and the basic response pattern (the canonical coefficients of the partial RDA, c_{dt}) to each treatment in relation to the reference group (T_low in our case) on the vertical axis (by definition, the reference group always has a c_{dt} of zero for every time; Cabecinha et al. 2024). PRC also displays the weights (b_k) on a separate vertical axis, which measure the affinity of a particular variable to the response pattern. The product $c_{dt}\ b_k$ indicates the value of a certain variable in a particular time and treatment. The significance of the model and the treatments was tested through Monte Carlo permutation tests (999 permutations). The PRC was fitted with function *prc* from package *vegan* (Oksanen et al. 2020).

We used linear mixed models (LMM) to test for treatment effects on water quality and total length of the fish. For the LMM, treatment, time and, when applicable, sex were considered as fixed effects, and tanks as random effects. LMM were obtained with function *lmer* of package *lme4* (Bates et al. 2015). *P*-values for the fixed and random effects were obtained with *lmerTest* package (Kuznetsova et al. 2017), while the marginal (R^2_m) and conditional (R^2_c) coefficients were computed with package *MuMIn* (Bartoń 2010). R^2_m represents the variance explained by the fixed effects, whereas R^2_c is the variance explained by the entire model, including fixed and random effects.

For variables that were only measured at the end of the experiment (i.e. population growth rate, fish abundance and condition and nutrient and chlorophyll *a* concentrations), we simply used conventional linear models (LM) to test for differences among treatments. In cases of significant treatment effects, the *glht* function from the *multcomp* package (Hothorn et al. 2008) was used to obtain Tukey multiple comparisons tests. Fish condition was assessed with total mass using total length as covariate and treatment and sex as categorical factors. An ANCOVA design with treatment × sex interaction tests the homogeneity of slopes of conventional ANCOVA (García-Berthou and Moreno-Amich 1993). Mosquitofish length or condition could not be studied in the Net treatment due to high mortality in this treatment. Population growth rate, fish total mass, total length, and nutrient and chlorophyll *a*

concentrations were $\log_{10}$-transformed for all analyses ($\log_{10}(x+1)$) for growth rate and $\log_{10}(x + a)$, for concentrations, where a was half the limit of quantification). All analyses were developed in the R environment (R Core Team 2021).

Results

Effects on water quality

Water quality (Suppl. material 1: tables S3, S4) varied markedly along the experiment and among treatments. The PRC model (Fig. 1) was significant ($F_{50, 183} = 1.54$, $P = 0.001$) and showed that time explained 53% of the variation in water quality variables, whereas treatment ($F_{5, 183} = 7.88$, $P = 0.001$) and its interaction with time explained 14%. The variables with strongest differences among treatments were turbidity and daytime dissolved oxygen percentage, which increased in the three treatments with mosquitofish at high densities (M_high, Mixed, and Net) (Fig. 1). Turbidity (and conductivity) clearly increased in these three treatments but not in the other three (Table 1, Fig. 2). By contrast, dissolved oxygen measured in the morning increased similarly in all treatments, but conditions remained rather hypoxic (Table 1, Suppl. material 1: fig. S3, table S3). Water temperature increased about 4 °C along the experiment, causing increased water conductivity and redox potential and a decrease in oxygen saturation despite its absolute increase; evaporation caused a decrease of 5 cm of water depth (Suppl. material 1: figs S3, S4).

At the end of the experiment, chlorophyll *a* concentration varied among treatments (ANOVA, $F_{5, 17} = 5.67$, $P = 0.003$) and was lowest with low fish densities and highest in the mosquitofish alone at high densities (Fig. 3). Nutrient concentrations did not vary clearly among treatments (ANOVAs, $P > 0.05$), except for Total P ($F_{5, 15} = 3.01$, $P = 0.044$), which was high particularly at the Net treatment and lowest at M_high (Suppl. material 1: fig. S5). Concentrations of TP, TOC, and TN were high, whereas nitrogen was in reduced forms due to the hypoxia (Suppl. material 1: table S4).

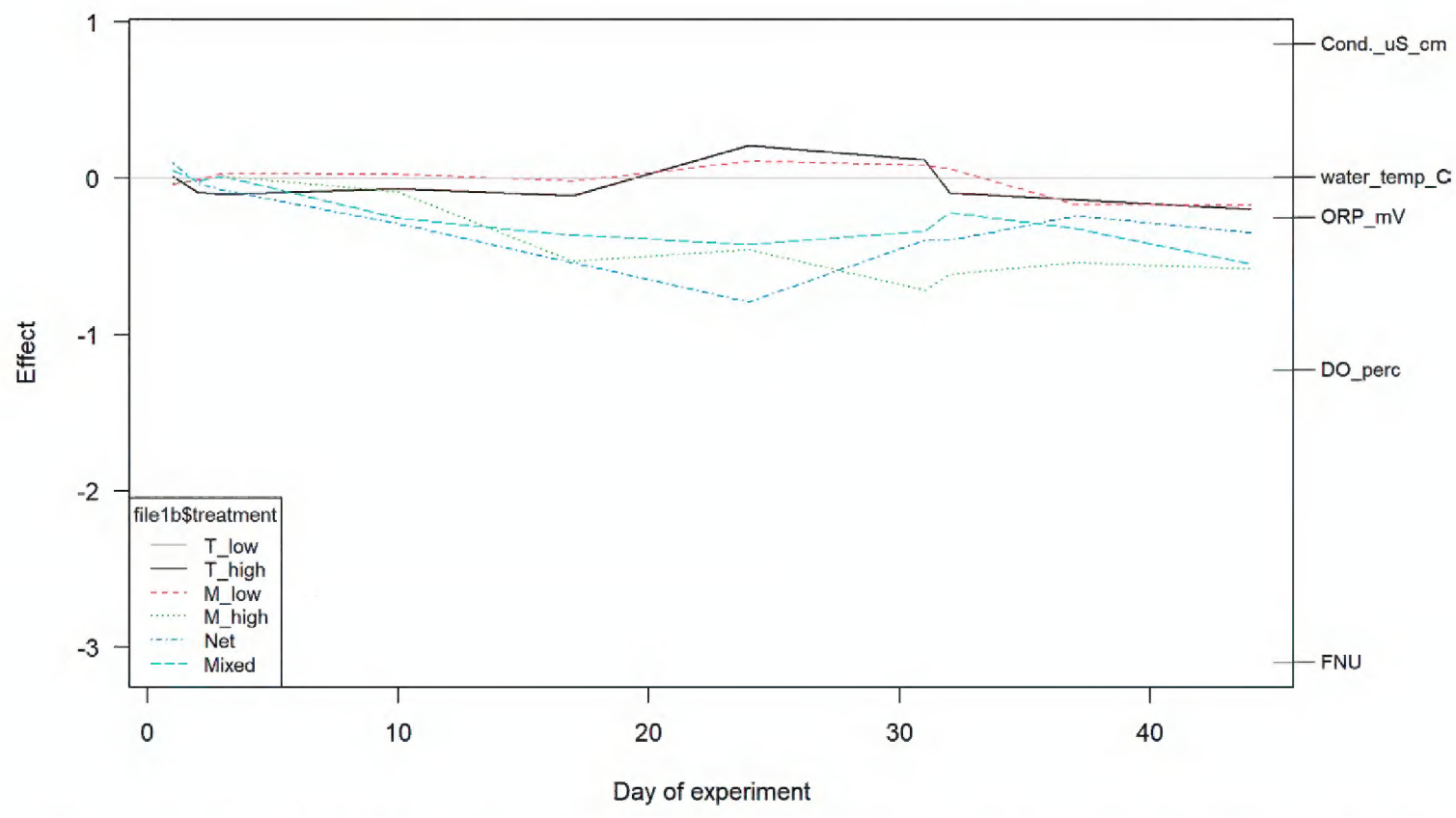

Figure 1. Principal response curve analysis of the effects of treatments on water quality along the experiment. The six treatments were: toothcarp at low density (T_low); mosquitofish at low density (M_low); toothcarp at high density (T_high); mosquitofish at high density (M_high); toothcarp and mosquitofish at high densities together (Mixed); and toothcarp and mosquitofish at high densities separated with a net (Net). The T_low treatment (grey line) was used as the reference for the analysis. The weights of the variables are displayed on the right vertical axis.

Table 1. Linear mixed models of the effects of treatments and time on water quality variables. Tanks were considered as random effects. The type III sums of squares, significance levels (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$), and marginal and conditional coefficients of determination are given.

	Turbidity	Dissolved oxygen	Conductivity
Marginal R^2	0.497	0.579	0.208
Conditional R^2	0.639	0.623	0.952
Treatment	62.0*	995	291
Time	188.3***	41769***	161393***
Treatment × time	405.5***	4726	25612***

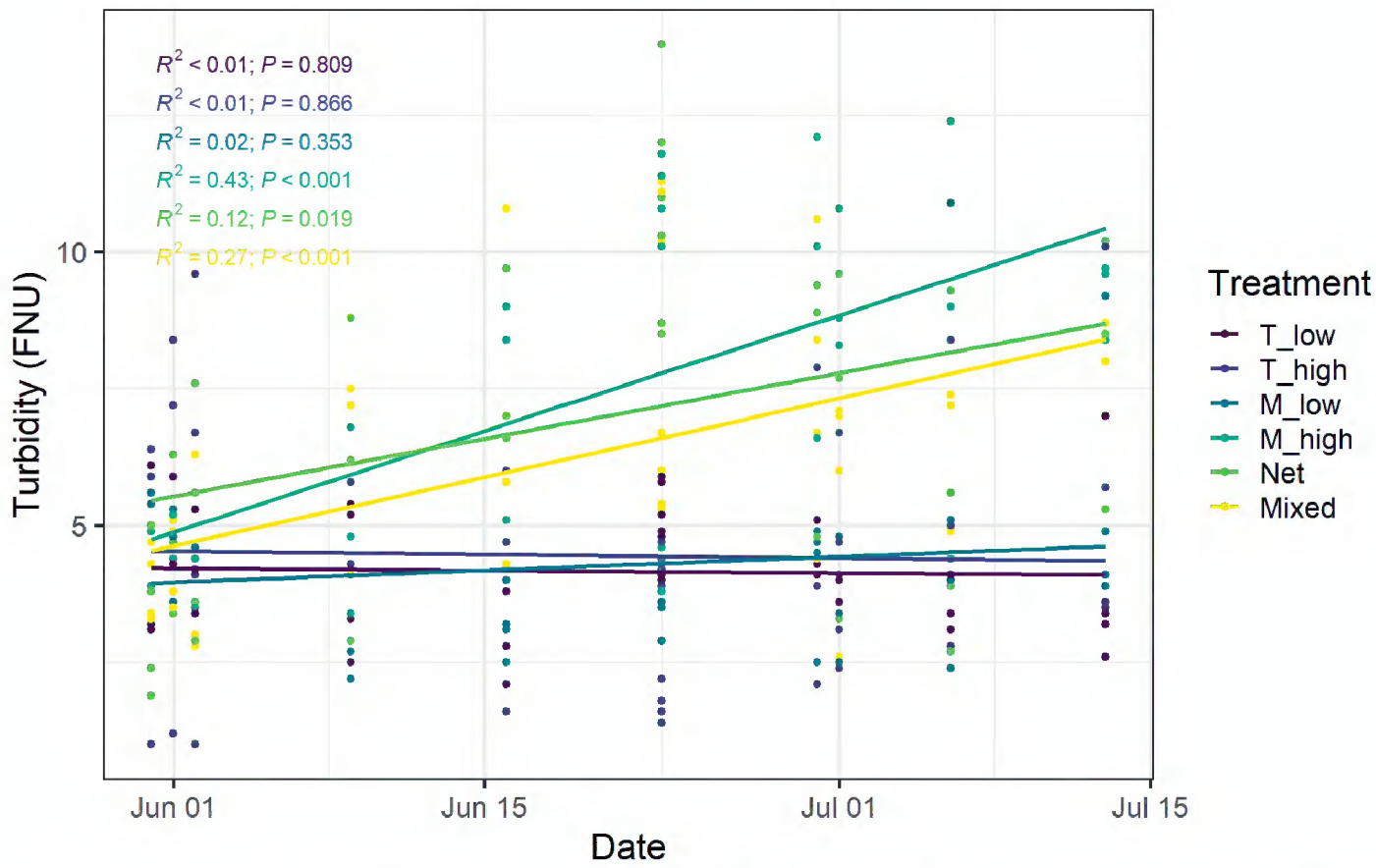

Figure 2. Variation in turbidity (FNU, Formazin Nephelometric Units) along the experiment by treatment. Linear regression functions and R^2 and P values are shown. The six treatments were: toothcarp at low density (T_low); mosquitofish at low density (M_low); toothcarp at high density (T_high); mosquitofish at high density (M_high); toothcarp and mosquitofish at high densities together (Mixed); and toothcarp and mosquitofish at high densities separated with a net (Net).

Effects on the density, size structure, and condition of fish

The population growth rates of both toothcarp (ANOVA, $F_{3,12} = 8.03$; $P = 0.003$) and mosquitofish ($F_{3,12} = 5.56$; $P = 0.012$) varied among treatments (Fig. 4). The abundance of immatures at the end of the experiment varied among treatments for mosquitofish ($F_{3,12} = 3.93$; $P = 0.036$) but only marginally for toothcarp ($F_{3,12} = 3.00$; $P = 0.073$) because immatures were only observed in the two low density treatments and were much more abundant for the invasive species. At low density treatments, toothcarp abundance remained stable or slightly increased, whereas that of mosquitofish increased enormously due to recruitment (Fig. 4). At higher densities, fish abundances generally declined due to the eutrophic, low-oxygen conditions. Tukey tests suggested that the growth rate of toothcarp was clearly different only between the T_low and Net and between T_low and Mixed treatments ($P < 0.05$). The results for mosquitofish were similar but Tukey tests were only marginally significant ($0.10 > P > 0.05$) because their growth rates were much more variable (Fig. 4). These results suggested that for toothcarp the effects of interspecific competition were more important than those of intraspecific competition, since there

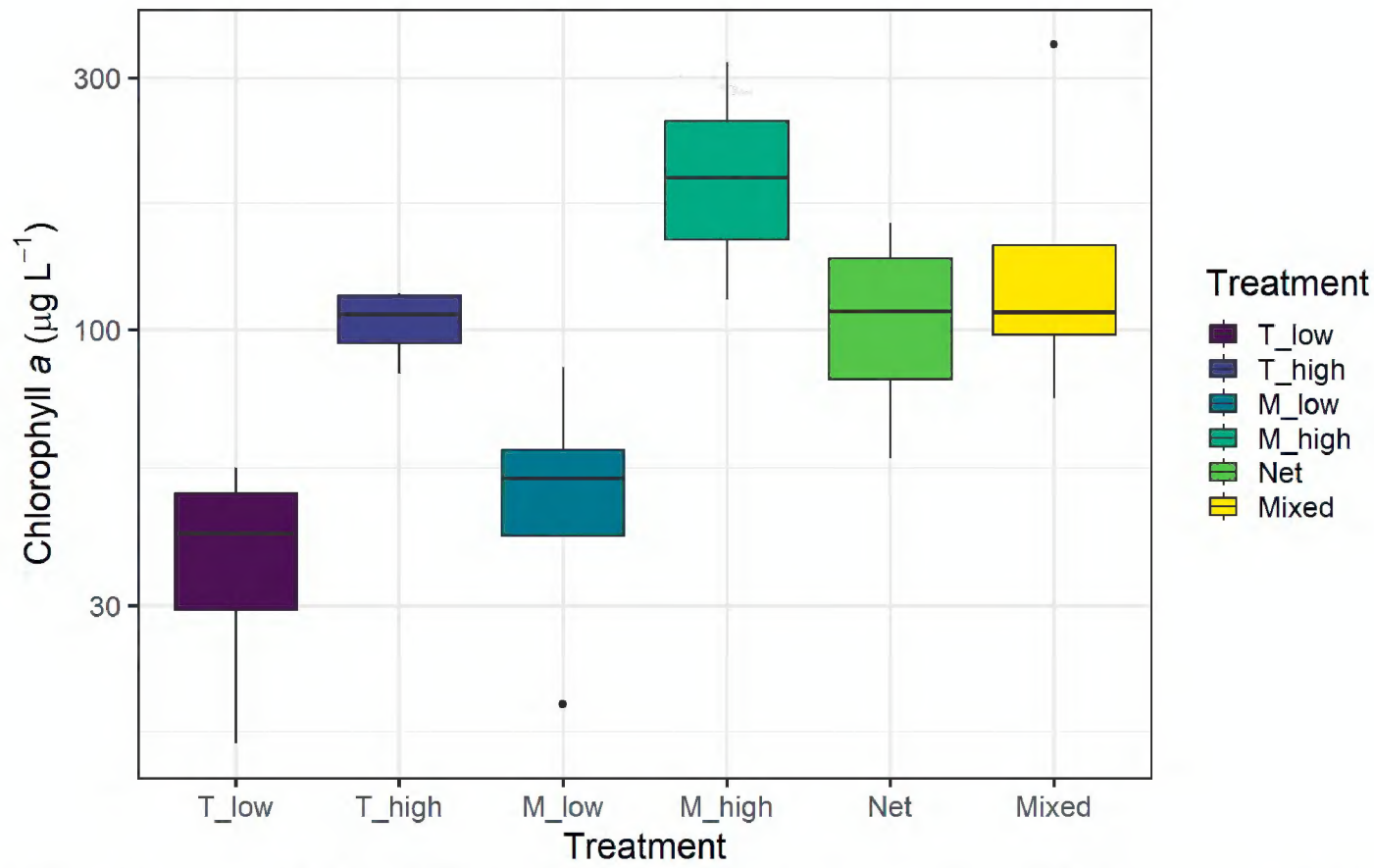

Figure 3. Variation of chlorophyll *a* concentration among treatments at the end of the experiment. The boxes represent the 25th and 75th percentiles, the line inside the box the median, and the error bars the minima and maxima except for outliers (black circles). See caption to Fig. 1 for the meaning of treatments.

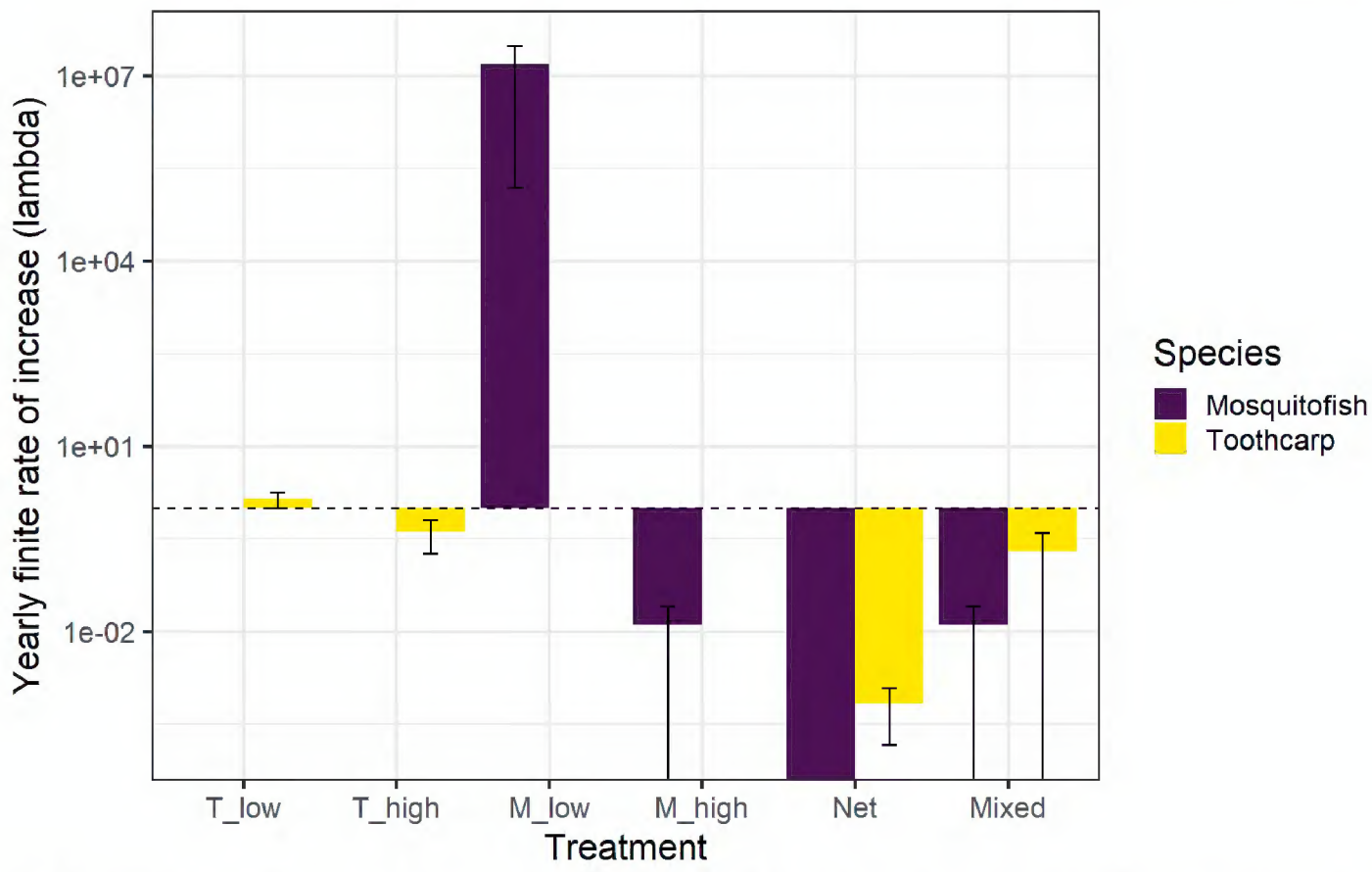

Figure 4. Population growth rates of mosquitofish and toothcarp at the end of the experiment by treatment. Values smaller than one (dashed line) indicate that fish abundance decreased. Bars are standard errors. See caption to Fig. 1 for the meaning of treatments.

was no clear difference between T_low and T_high (P = 0.053). Besides, the role of interference competition and predation by mosquitofish was small compared to resource competition as toothcarp abundance decreased more in Net treatment (Fig. 4), where only exploitation competition was acting.

The differences in size structure from the start to the end of the experiment, which might be due to individual growth, size-dependent mortality or recruitment, varied among treatments for both toothcarp and mosquitofish (treatment × time, Table 2, Fig. 5, Suppl. material 1: tables S1, S2). Toothcarp, particularly females, generally increased in mean size at the end of the experiment, except when together with mosquitofish (Mixed treatment) (Fig. 5A).

Table 2. Linear mixed models of the effects of treatments and time on the total length of fish species. Tanks were considered as random effects. The type III sums of squares, significance levels (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$), and marginal and conditional coefficients of determination are given.

	Toothcarp	Mosquitofish
Conditional R^2	0.189	0.703
Marginal R^2	0.497	0.687
Treatment	89.4	2.6
Time	492.7***	10.2**
Treatment × time	155.5*	13.6***
Sex	1138.6***	372.0***

By contrast, mosquitofish mean size remained quite stable, except for a marked decrease in the low-density treatment (T_low) (Fig. 5B), likely due to recruitment as suggested by population growth rate (Fig. 4).

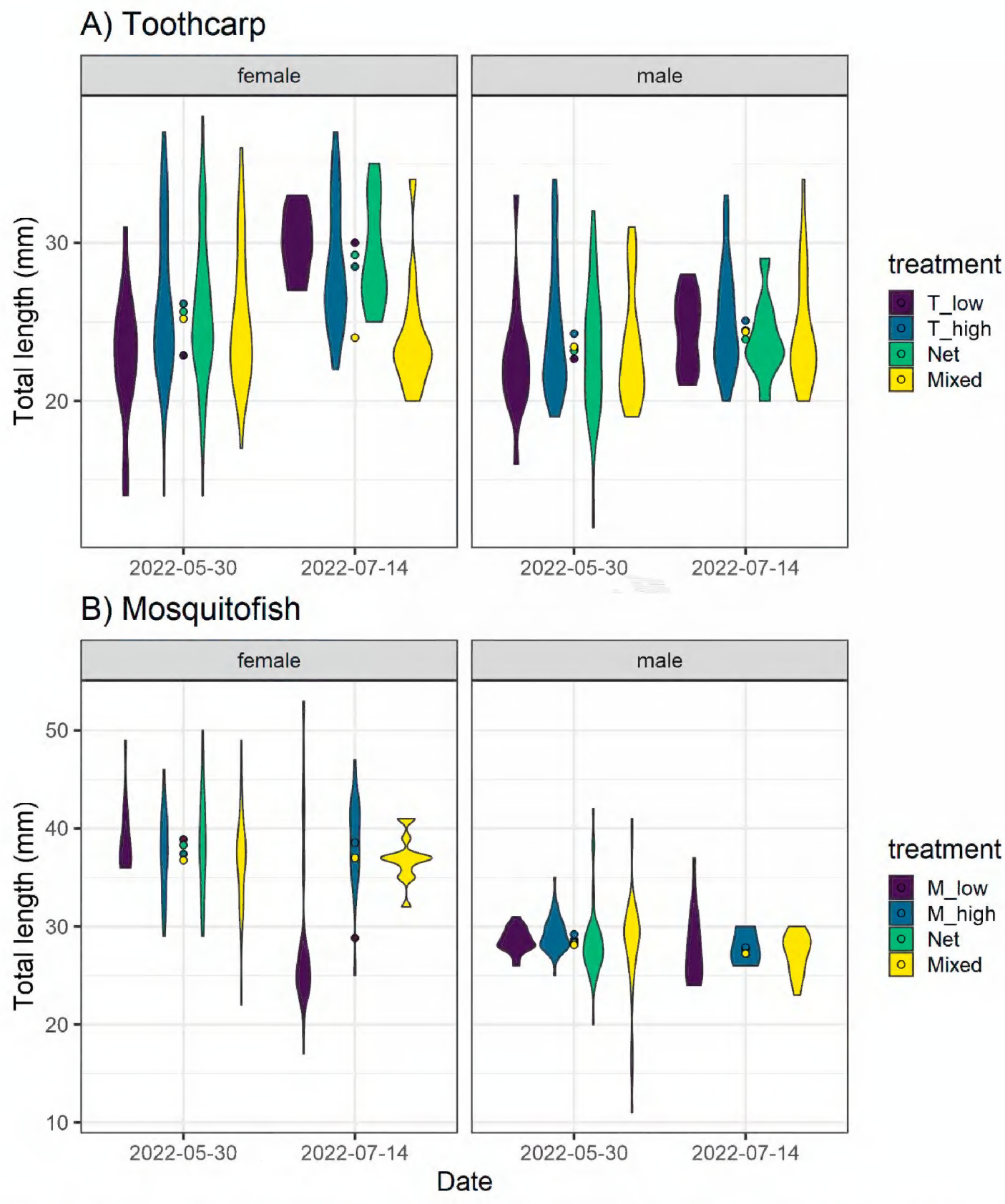

Figure 5. Violin plots with the kernel probability densities of the total length of toothcarp (**A**) and mosquitofish (**B**) by sex at the start (30 May 2022) and the end (14 July) of the experiment. Circles show the average per treatment. See caption to Fig. 1 for the meaning of treatments.

At the end of the experiment, the individual condition of fish (mass after adjusting for length) varied markedly among treatments for both species (ANCOVAs, P values < 0.001), with no clear interactions of treatment with fish size (i.e. similar slopes) or sex (P values > 0.05). The only significant differences in the size-adjusted means of mass (Tukey tests, $P < 0.05$) were between T_low and T_high, indicating intraspecific competition, and between Mixed and T_high, indicating interspecific effects of the invasive fish on the threatened species (Fig. 6). For mosquitofish, condition was higher in the low-density treatment (M_low) than in the other three treatments, particularly for females (Fig. 7), indicating that intraspecific competition was more important than the interspecific effects of toothcarp.

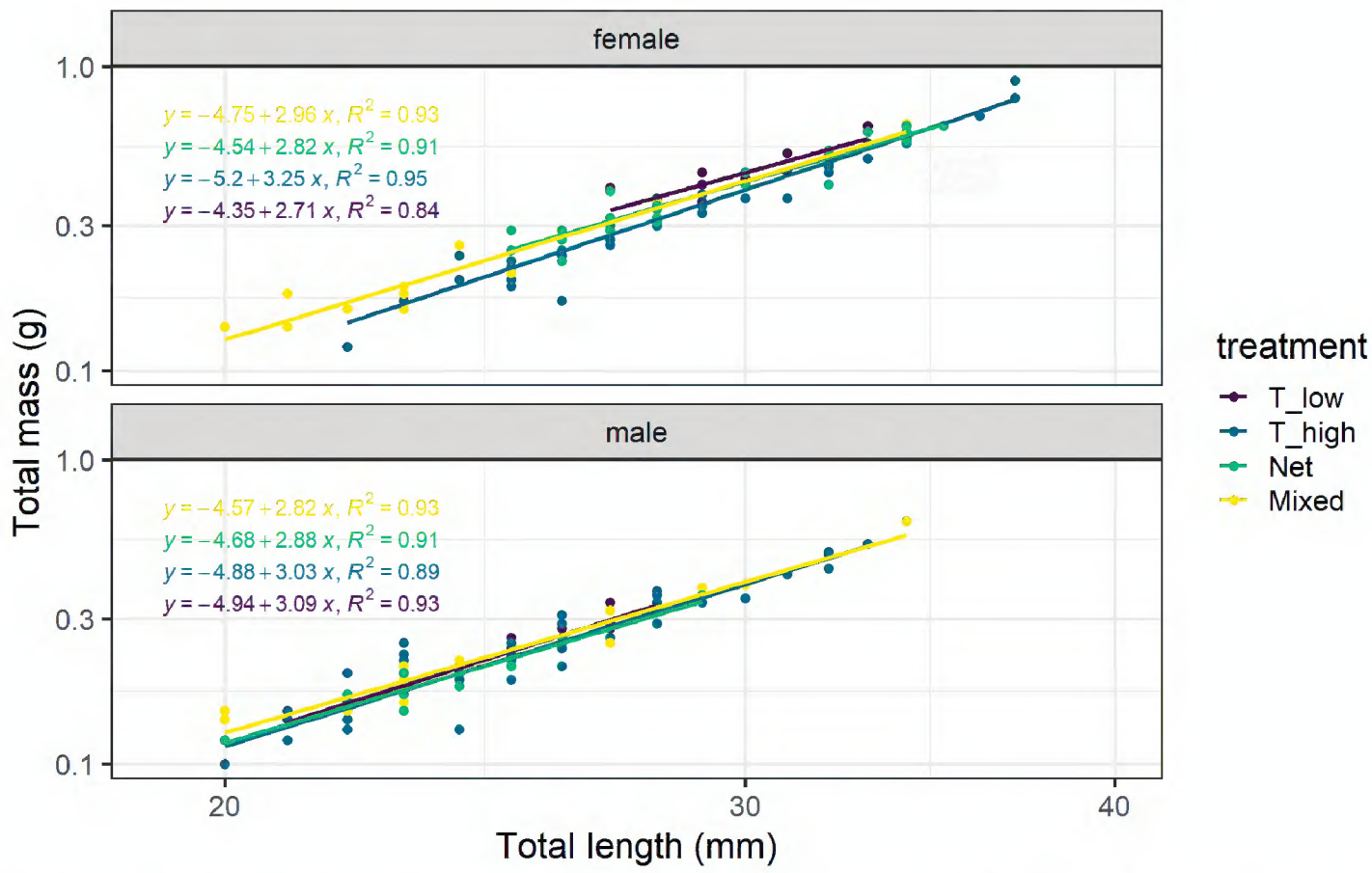

Figure 6. Relationship between total mass and total length (condition) for toothcarp at the end of the experiment by treatment and sex. The linear regression functions are shown. See the caption to Fig. 1 for the meaning of treatments.

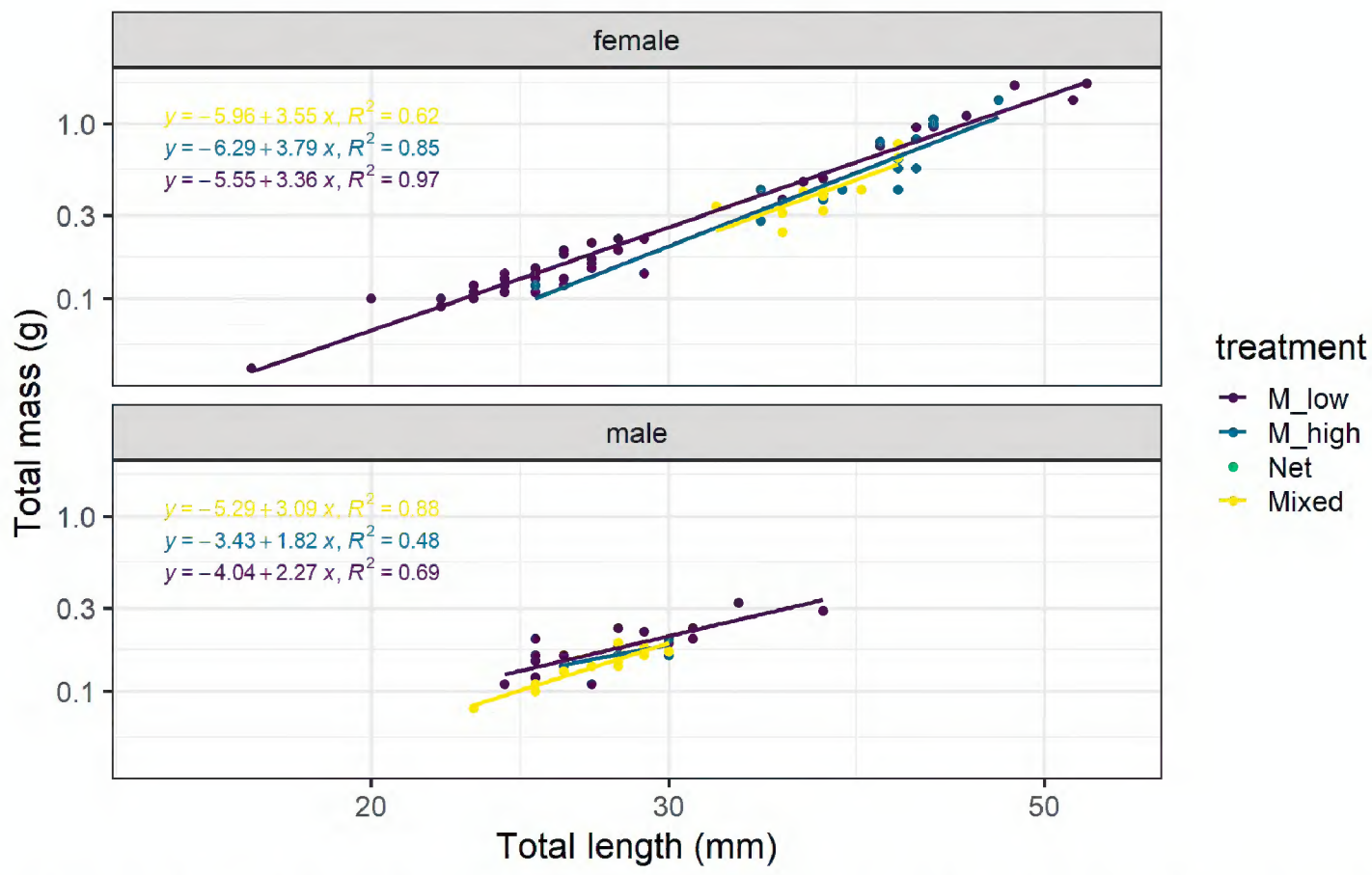

Figure 7. Relationship between total mass and total length (condition) for mosquitofish at the end of the experiment by treatment and sex. The linear regression functions are shown. See the caption to Fig. 2 for details of treatments.

Discussion

Stronger effects of mosquitofish on water quality

Our mesocosm experiment demonstrated clear treatment effects on water quality for both fish species but stronger for mosquitofish. At high fish densities, turbidity, chlorophyll *a* concentration and daytime dissolved oxygen percentage increased, whereas total phosphorus decreased. These results agree with the trophic cascade that has been well shown for *G. affinis* (Hurlbert et al. 1972; Hurlbert and Mulla 1981), but often not observed for *G. holbrooki*, despite clear depletions of zooplankton (Angeler et al. 2002; Romo and Villena 2005; Cardona 2006). The introduction or increase in abundance of mosquitofish tends to markedly affect the species composition of zooplankton, particularly decreasing the abundance of cladocerans and secondarily cyclopoid copepods and increasing rotifers (Hurlbert and Mulla 1981; Miracle et al. 2007). These often cascade down to the phytoplankton, as in our experiment, with increases in turbidity and chlorophyll a concentration, and thus clear ecosystem effects. A trophic cascade has also been shown before for the toothcarp (Compte et al. 2011, 2012) but our results show that it is stronger for mosquitofish, likely because the latter species preys more on water column invertebrates rather than more benthic prey (García-Berthou 1999; Alcaraz and García-Berthou 2007a) and is more voracious (Rehage et al. 2005; Alcaraz et al. 2008; Carmona-Catot et al. 2013).

The lack of top-down effects on phytoplankton in some previous studies of *G. holbrooki* (Angeler et al. 2002; Romo and Villena 2005; Cardona 2006), in contrast to the present one, might be due to a number of reasons. Top-down control of phytoplankton by fish is frequent but variable and depends on a number of factors, including differences in trophic state, importance of macrophytes, or methodological aspects of the studies (Carpenter et al. 2001; Benndorf et al. 2002; Jeppesen et al. 2003; Su et al. 2021). Most experiments with the two study species have been performed in shallow, rather eutrophic conditions, such as the ones in our study, which are the typical habitat of these fishes. Some authors suggest that the control of phytoplankton by fish increases with eutrophication (Benndorf et al. 2002; Su et al. 2021), whereas others suggest that it decreases (Jeppesen et al. 2003). The effects of *G. affinis* seem indeed to be amplificated with increased nutrient availability (Preston et al. 2018). The control of zooplankton, but not phytoplankton, by *G. holbrooki* in an enclosure experiment was attributed to the bottom-up control by submerged macrophytes (Cardona 2006), which were scarce in our mesocosms. Control of phytoplankton has been suggested to be small in clear states of shallow lakes dominated by macrophytes (Benndorf et al. 2002). Field experiments might provide more realistic results than mesocosm tanks (Schmitz 2008), such as the ones used in our study, but have more ethical limitations and are not free of artefacts (see e.g. Compte et al. 2012).

The effects of invasive mosquitofish on the threatened toothcarp

The results of our experiment showed that: i) the interspecific effects of mosquitofish on toothcarp were more important than those of intraspecific competition; and ii) that the invasive species produced effects on population growth rates, size structure, and fish condition (mass-length relationship) of toothcarp. This confirms previous observational and experimental evidence that this invasive fish partly explains the decline of the threatened, endemic species (Rincón et al. 2002; Alcaraz and García-Berthou 2007b; Carmona-Catot et al. 2013; Magellan and

García-Berthou 2015, 2016). Our study was performed in shallow (40–45 cm), eutrophic conditions, which are representative of the habitat of the study species. However, these effects are known to be context-dependent and increase with warmer temperatures and less saline waters (Alcaraz et al. 2008; Carmona-Catot et al. 2013), so further studies are needed to understand this generalization and the long-term coexistence of these two species. Our study also suggests that low-oxygen, eutrophic conditions, which are increasingly prevalent with warming temperatures and eutrophication of coastal lagoons, limit the abundance and population growth rate of these two fish species.

Regarding the impact mechanism, the Net treatment, where only resource competition was possible, displayed the lowest population growth rate of toothcarp, suggesting that resource competition was more important for this response variable than predation on eggs or young-of-the-year fish or interference competition (agonistic interactions). By contrast, size structure and condition of toothcarp decreased more strongly in the Mixed treatment, where the three interaction types were possible. In agreement, Rincón et al. (2002) also observed that young-of-the-year toothcarp were 8% smaller in size in mesocosms with mosquitofish and suggested that this was due to reduced growth rather than size-selective predation.

Therefore, our results suggest that at least two (or three) mechanisms are acting, in agreement with previous literature. Previous studies emphasized resource and interference competition (Carmona-Catot et al. 2013; Magellan and García-Berthou 2015, 2016), whereas only Rincón et al. (2002) also observed predation on young-of-the-year toothcarp, reducing offspring by 70%. In fact, these mechanisms can interact, since the aggressive behaviour of mosquitofish enhances toothcarp stationary behaviour, thus decreasing its feeding activity to the advantage of the invasive species (Caiola and de Sostoa 2005). Fish microhabitat use (and thus feeding rates) of toothcarp might also be affected by interference competition from mosquitofish. It also makes sense that more direct interactions such as predation and interference competition affected more variables that can respond rapidly, such as size structure and individual condition, whereas a more indirect interaction such as resource competition, which needs reduction of resource availability, affected more population growth rate (Eccard et al. 2011).

At low initial fish densities, population growth rate of mosquitofish was enormous and orders of magnitude larger than that of toothcarp. Both fish species are small and short-lived, they mature at a few weeks of age, reproduce during a long season in spring and summer, and mostly die when they are a few months old in winter (Fernandez-Delgado et al. 1988; Fernández-Delgado 1989; García-Berthou and Moreno-Amich 1992, 1993). However, the invasive species is a live-bearer, which produces large numbers of young per litter (30–500), approximately every 3–5 weeks (Fernández-Delgado 1989; Fernández-Delgado and Rossomanno 1997). By contrast, the toothcarp lays multiple batches of a few large eggs. The extreme increases in abundance of mosquitofish are well known (Matthews and Marsh-Matthews 2011) and are probably important in explaining their ecosystem effects and their overall impacts on native species, which are likely more important in summer and oligohaline waters (Alcaraz et al. 2008; Carmona-Catot et al. 2013). These ecosystem effects (e.g. increases in turbidity) might also affect species interactions (e.g. feeding rates, reproductive behaviour, or aggressions) and thus underscore the difficulty of predicting the environmental impacts of mosquitofishes and other invasive species.

Overall, our results reinforce the evidence that eastern mosquitofish have significant impacts on water quality and threatened, endemic toothcarps. The combination of resource competition, interference, and predation suggests that the impact on native cyprinodontiform fishes can be important, including under eutrophic and low-oxygen conditions, which are expected to become more prevalent with global change. This study also illustrates how the use of mesocosms with and without meshes helps to understand if the impact mechanism of aquatic invasive species is through resource competition or through more direct interactions (e.g. predation and agonistic behaviour). Our findings also highlight the importance of management strategies aimed at preventing the introduction and spread of invasive species and promoting the conservation of endemic species in aquatic ecosystems.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

Applicable national legislation for the care and use of animals were followed and the experimental protocol was approved by Animal Ethics Committee of the Autonomous University of Barcelona (ref. CEEAH 6690).

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization: EGB, LZ. Data curation: EGB, IGL. Formal analysis: EGB, IGL. Funding acquisition: EGB, ID. Investigation: LZ, IGL. Methodology: AEM, EGB, ID, CFD, IGL, LZ. Project administration: EGB, ID. Resources: PR. Supervision: EGB, LZ. Writing - original draft: EGB, IGL. Writing - review and editing: ID, PR, AEM, CFD, LZ.

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Data availability

All of the data that support the findings of this study are available at https://doi.org/10.6084/m9.figshare.29491010.v3.

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Supplementary material 1

Supplementary information: figs S1–S5, tables S1–S4

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Data type: docx

Explanation note: figs S1–S5, tables S1–S4.

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